

Four new species of trypanoplasms from the fresh water fishes of the genus *Mystus* in Maharashtra

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Abstract. Four new species of haemoflagellates of the genus *Trypanoplasma*, Laveran and Mesnil, 1901 viz *T. Krishnamurthyi*, *T. cavacii*, *T. vidyai* and *T. seenghali* are described from the fresh water fishes of the genus *Mystus* in Maharashtra.

Keywords. Haematozoa; haemoflagellates; *Trypanoplasma*.

1. Introduction

Biflagellate organisms of the genus *Trypanoplasma* Laveran and Mesnil 1901 from fresh water fishes have been recorded from various parts of the world. The major reviews of this group are those of Pavlovskii (1964), Becker (1970, 1977) and Lom (1979). However, this group received little attention in India. Mandal (1979) and Joshi (1982) are the only workers who have described one and two species respectively. The present contribution is the third of a series on this group in Maharashtra.

2. Material and methods

The material for this investigation was obtained from two rivers in two different localities, namely Purna and Aurangabad in Maharashtra. The fishes were brought alive to the laboratory for examination or were examined on the spot itself using a field microscope. Smears were made from the blood obtained from the heart and no anti-coagulant was used. The smears were air-dried, fixed in acetone-free methyl alcohol for 8–10 min and stained with Giemsa's stain diluted with phosphate buffer. The drawings were using a Leitz camera lucida at a magnification of about 2000 \times . The photo-micrographs were taken with Leica M-3 camera. The identification of the fish hosts was based on Day (1875), Shrivastava (1968) and Jhingran (1982). The slides of the type material are deposited in the Protozoology Section, Department of Zoology, Marathwada University, Aurangabad, India.

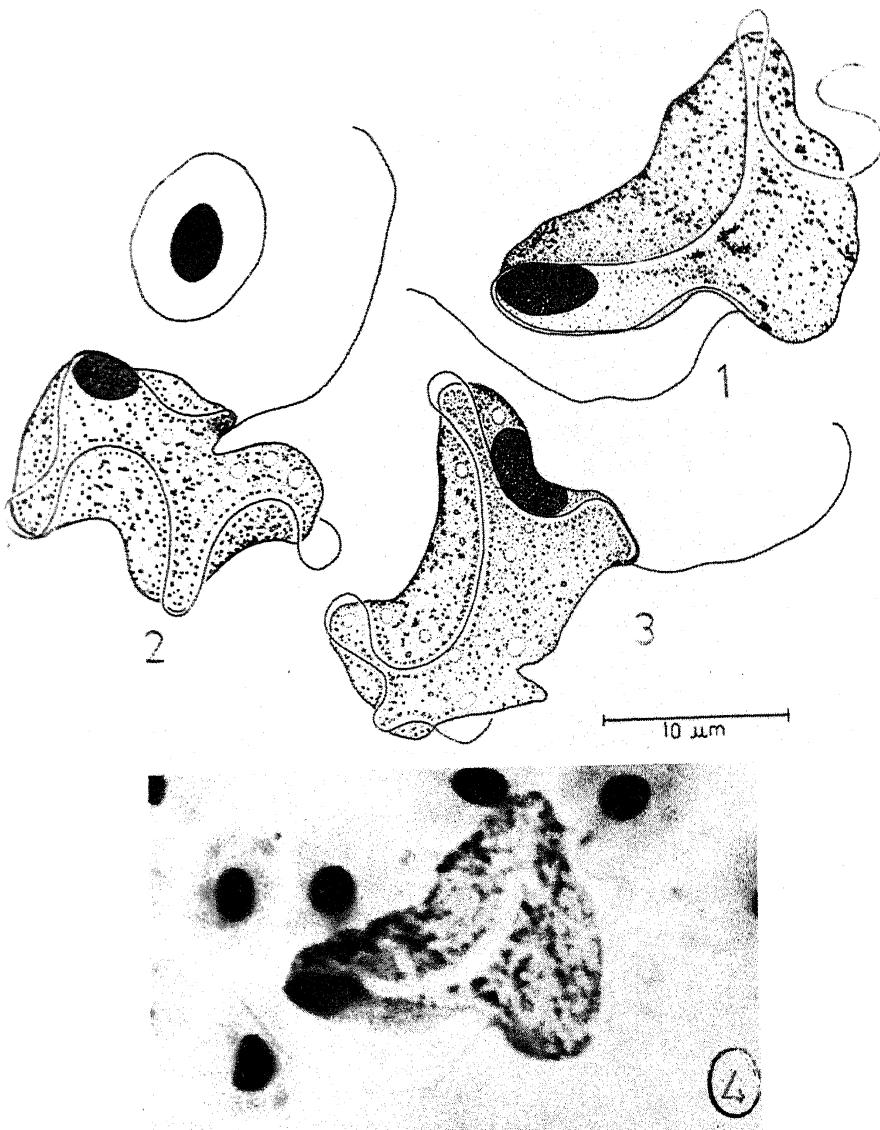
3. Observations and discussion

3.1 *Trypanoplasma Krishnamurthyi* sp. nov. figures 1–4

Host: *Mystus cavacius* Hamilton.

Locality: River Purna, Parbhani Dist., Maharashtra, India.

Site of infection: Blood.



Figures 1-4. *Trypanoplasma krishnamurthyi* sp. nov. from *M. cavacius*. 1-3. Camera lucida drawings. 4. Photomicrograph.

This trypanoplasm was present in two out of the four fishes (*Mystus cavacius*) examined. The infection was moderate in both the cases.

Morphology

Cell body: The body of the trypanoplasm is short, broad and often irregular in shape.

Cytoplasm: The cytoplasm is vacuolated and granular, but does not show homogeneous appearance because of varying patterns of granulation in different regions.

Nucleus: The nucleus is dorsal and very characteristic in having a distinctly ovoidal shape (length: width = 2:1) consistently. This is the only species found during the present study having a consistent nuclear shape.

Kinetoplast: The kinetoplast is absent which is extremely characteristic, as no dis-kinetoplastic trypanoplasm has ever been described so far, from fresh water fishes.

Flagella and undulating membrane: The two flagella arise from the kinetosomes which

are distinct and rod-like. The anterior flagellum is about as long as the body. The posterior flagellum forms an 'S' configuration as it runs over the body. The free trailing portion of the posterior flagellum is long, being slightly less than the body length.

There is no clear evidence of the presence of an undulating membrane.

The details of dimensions of the trypanoplasm are given in table 1.

This is the first trypanoplasm to be described from this host species and the third from a fish of the genus *Mystus* in India, the earlier records being those of *T. indica* from *M. vittatus* and *T. mysti* from *M. aor*.

A comparison of this species with *T. indica* (Mandal 1979) shows that it is distinctly smaller in size, measuring $15.53 - 28.24 \times 5.17 - 16 \mu\text{m}$ ($19.89 \times 10.13 \mu\text{m}$) as against $25 - 30.5 \times 6 - 10.5 \mu\text{m}$ ($28.5 \times 8 \mu\text{m}$) in the present form. This form is unique in lacking a kinetoplast, while *T. indica* has a conspicuous one. It also has a much longer trailing flagellum than *T. indica*.

It is marked off from *T. mysti* Joshi, 1982, by its smaller size, ($19.89 \times 10.13 \mu\text{m}$ as against $28.2 \times 10.9 \mu\text{m}$) larger nucleus and distinctly longer flagella. The monomorphic nature of this trypanoplasm distinguishes it from *T. atti* Joshi, 1982 which exists in two forms. The two species are also marked off by differences in morphology and morphometrics.

The only other record of trypanoplasm from fishes of the family Bagridae is *T. pseudobagri* Chang, 1964 from *Pseudobagrus fulvidraco* in China. Unfortunately neither its description nor any information about this species could be procured by the author and hence no comparison could be made.

The only trypanoplasm without a kinetoplast recorded earlier is *T. beckeri* Burreson, 1979. That species was described from a marine fish *Scorpaenichthys marmoratus* of family Cottidae in the United States, while the present species is from a fresh water fish of the family Bagridae in India. The body of the present form is relatively short and broad ($19.89 \times 10.13 \mu\text{m}$) as contrasted with the extremely long and twisted body of *T. beckeri* ($109 \times 6.5 \mu\text{m}$).

In view of its distinctness, this species is considered new to Science and designated *Trypanoplasma Krishnamurthyi* sp. nov. after Dr R Krishnamurthy of the Marathwada University, Aurangabad, in grateful appreciation of his active guidance throughout the course of this work.

Table 1. Body dimensions of *T. Krishnamurthyi* sp. nov. from *M. cavacius* (based on 50 forms).

Particulars (μm)	Minimum	Maximum	Average
Length of cell body	15.53	28.24	19.89
Width of cell body	5.17	16.00	10.13
Length of nucleus	3.28	11.29	6.91
Width of nucleus	1.41	6.12	3.08
Length of kinetoplast	—	—	—
Width of kinetoplast	—	—	—
Length of anterior free flagellum	10.35	30.13	22.52
Length of posterior free flagellum	11.29	27.30	18.38

3.2 *Trypanoplasma cavacii* sp. nov. figures 5–10

Host: *Mystus cavacius* Hamilton.

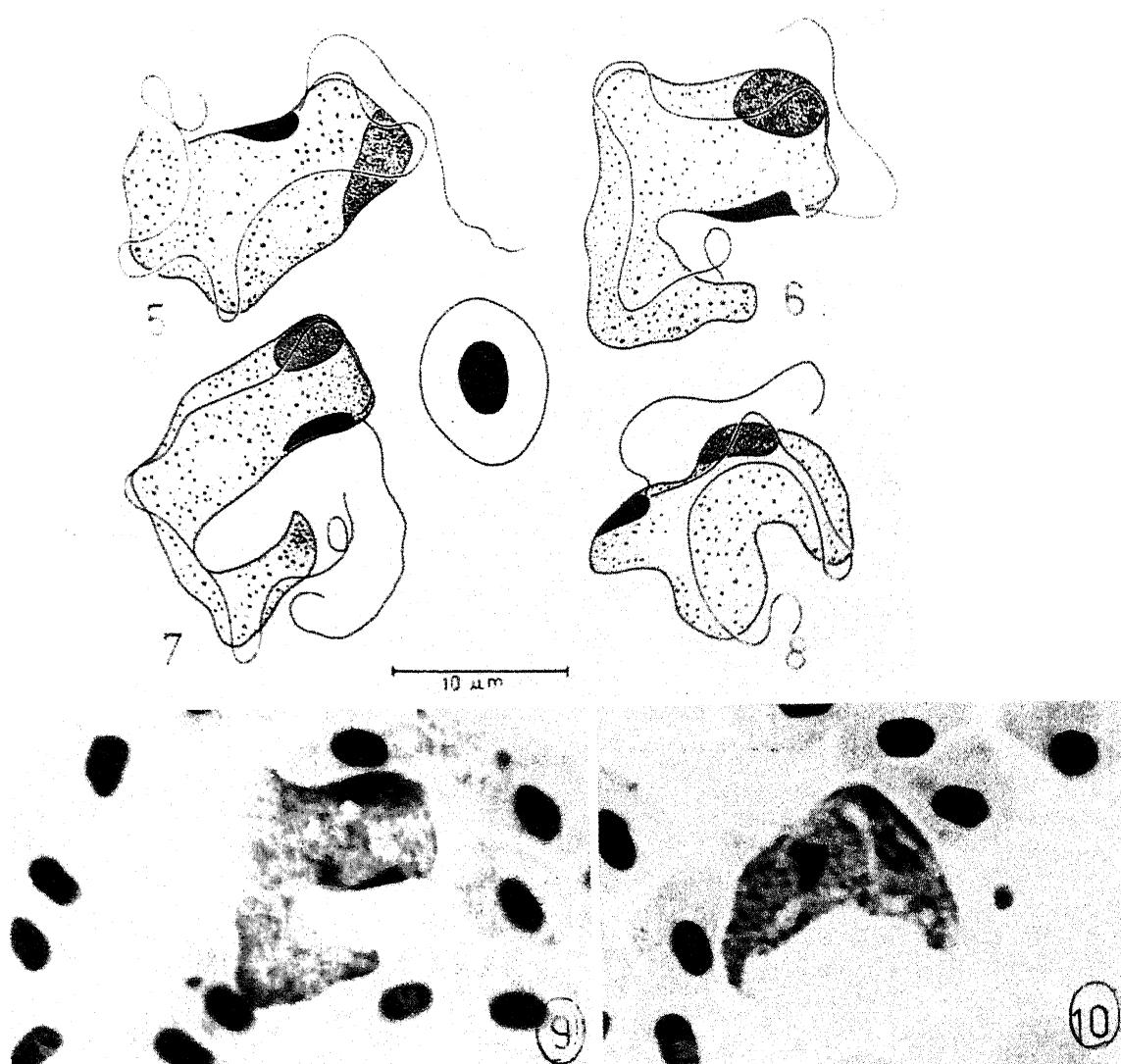
Locality: Kham river, Waluj, Aurangabad, Maharashtra, India.

Site of infection: Blood.

This trypanoplasm was found as the only species in 10 out of the 51 fishes (*M. cavacius*), examined. The infection was light in all the cases and a few dividing forms were also observed (figure 10).

Morphology

Cell body: The body of the trypanoplasm is variable in shape. The elongated forms have a 'J' shaped configuration (figures 6, 7) with the anterior part being broad and forming the longer arm and the posterior third being relatively narrower and forming the shorter arm. In other cases, the body is short, broad and almost straight (figure 5).



Figures 5–10. *Trypanoplasma cavacii* sp. nov. from *M. cavacius*. 5–8. Camera lucida drawings. 9. Photomicrograph of 'Trophozoite'. 10. Photomicrograph of dividing form.

Cytoplasm: The cytoplasm is vacuolated and shows distinctly coarse chromophilic granules, often abundant near the posterior end (figures 6, 7).

Nucleus: The nucleus is ovoid (figures 6, 7) to elongated (figure 8) and placed dorsally, close to the anterior end.

Kinetoplast: The kinetoplast is relatively short ($L:W = 2-4:1$) distinctly triangular (figure 6) or fusiform (figure 7) in the elongated specimens and slightly curved and somewhat crescentic in the short forms (figure 5). The kinetoplast is ventral in position.

Flagella and undulating membrane: The two flagella arise from the kinetosomes, which are placed just anterior to the kinetoplast. The anterior flagellum becomes free from the body soon after the origin, and it is about as long as body or slightly more. The posterior flagellum forms 2-3 distinct folds along the length of the body, before becoming free. The free trailing part is about as long as the body.

There is no clear evidence of an undulating membrane.

The details of the dimensions of the trypanoplasm are given in table 2.

This trypanoplasm is described from the same host (i.e. *M. cavacius*) as the preceding species i.e. *T. Krishnamurthyi* sp. nov. It is easily distinguished from the earlier species by the presence of a distinct kinetoplast, its slightly smaller size ($17.63 \times 9.58 \mu\text{m}$ as against $19.89 \times 10.13 \mu\text{m}$) and by the presence of distinctly coarse chromophilic granules in the cytoplasm. The present species is much smaller in size than *T. indica* ($17.63 \times 9.58 \mu\text{m}$ as against $28.5 \times 8 \mu\text{m}$), but has a nucleus and kinetoplast which are only slightly smaller. It also has a conspicuously longer trailing flagellum ($16.94 \mu\text{m}$) compared with *T. indica* ($10.5 \mu\text{m}$).

Compared with four other species of trypanoplasms described from fresh water fishes in Maharashtra, i.e. *T. saranae*, *T. lomi* and *T. solapurensis*, Wahul (under publication) and *T. quadrii*, Krishnamurthy and Wahul (under publication) it is marked off by differences in the shape and size of the body and kinetoplast, the pattern of distribution of chromophilic granules in the cytoplasm and by a much longer trailing flagellum. Further it is distinguished from *T. quadrii* by its monomorphic nature.

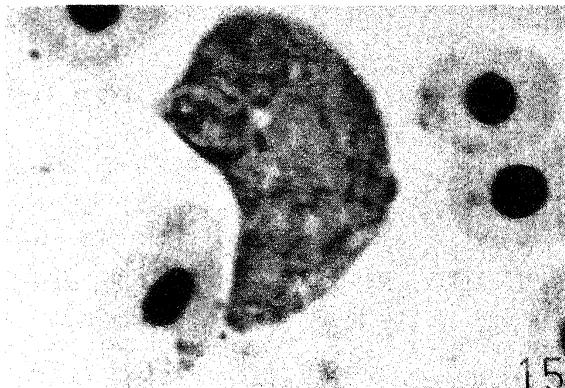
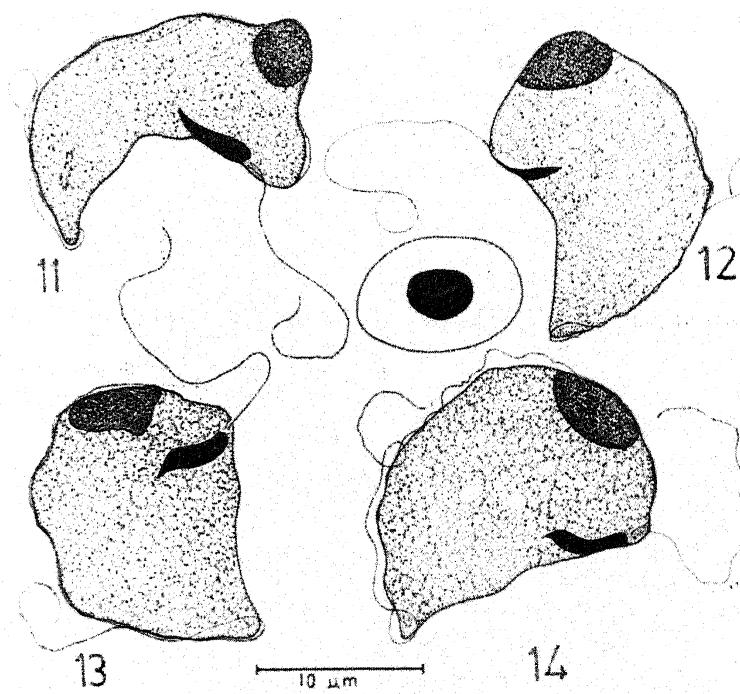
This species has distinctly smaller body dimensions than *T. mysti* but has a larger nucleus, a better developed kinetoplast and distinctly longer flagella. This species is also much smaller than *T. atti* and is typically monomorphic besides having distinctly longer flagella.

Table 2. Body dimensions of *T. cavacii* sp. nov. from *M. cavacius* (based on 25 forms).

Particulars (μm)	Minimum	Maximum	Average
Length of cell body	15.06	20.24	17.63
Width of cell body	6.59	14.59	9.58
Length of nucleus	4.23	8.47	6.25
Width of nucleus	1.41	4.23	2.65
Length of kinetoplast	2.11	6.12	4.33
Width of kinetoplast	0.47	3.29	1.43
Length of anterior free flagellum	13.65	23.06	20.41
Length of posterior free flagellum	12.17	21.18	16.94

It has an overlapping range in body length with *T. borelli*, Laveran and Mesnil (1901), *T. cataractae* Putz (1972), and *T. cyprini*, Plehn (1903) but is clearly much broader. It has a larger nucleus than *T. borelli* and *T. cataractae* and a smaller kinetoplast than *T. cyprini*. It has an overlapping but narrower length range with *T. varium*, Leger (1904), but is contrasted from it by its definite shape, smaller kinetoplast and longer trailing flagellum. It is marked off from all the other species by its size, being smaller than *T. abramidis* Brumpt (1906), *T. barbi*, Brumpt (1906), *T. acipenseris*, Ioff et al (1926) and *T. guernei*, Brumpt (1906) and larger than *T. makeevi*, Achemerov (1959), *T. salmositica*, Katz (1951), *T. markewitschi*, Schapowal (1953) and *T. pseudocaphirhynchi*, Ostroumov (1949).

In light of the above discussion, it is considered new and named *Trypanoplasma cavacii* sp. nov. after the specific name of the host in which it was found.



Figures 11-15. *Trypanoplasma vidyai* sp. nov. from *M. seenghala*. 11-14. Camera lucida drawings. 15. Photomicrograph.

3.3 *Trypanoplasma vidyai* sp. nov. figures 11–15

Host: *Mystus seenghala* Sykes.

Locality: River Purna, Parbhani Dist., Maharashtra, India.

Site of infection: Blood.

This trypanoplasm was found in 8 (*Mystus seenghala*) out of the 28 fishes examined. Out of the 8 infected fishes it occurred alongwith another species of *Trypanoplasma* (*T. seenghali* sp. nov.) and a species of *Trypanosoma* in one while in the other seven it occurred with another species of *Trypanosoma*. The infection was moderate in all cases.

Morphology

Cell body: The body of the trypanoplasm is short, broad and stumpy (L:W 2:1) with a distinctly convex dorsal margin and a straight (figures 13–14) or curved (figures 11, 12) concave ventral margin. The anterior end is broad and rounded while the posterior is bluntly conical (figure 14).

Cytoplasm: The cytoplasm is highly vacuolated (figure 14) and stains homogeneously and intensely.

Nucleus: The nucleus is spherical (figure 11) to ovoidal (figure 14) and lies along the dorsal margin in the anterior third of the body.

Kinetoplast: The kinetoplast is relatively short, broad and variable in shape, the L:W ratio varying from 2:1 to 4:1. The width of the kinetoplast is not uniform and the posterior end is generally pointed. In most cases it runs partly along the ventral margin and turns away from the margin into the cytoplasm (figures 11, 14).

Flagella and undulating membrane: The two very delicate flagella arise from the kinetosomes which are placed just anterior to the kinetoplast. The anterior flagellum is relatively long, being one and one-third times the body length. The posterior flagellum is extremely characteristic, running along or very close to the dorsal surface of the body, almost up to the posterior tip. In most cases there are hardly any undulations visible (figure 11) and where they are present, the undulations are numerous, very small and shallow attaching the flagellum to the body surface at several points (figures 12–14). It is also characteristic by its free trailing part, which in almost all cases, recurses and extends forward along the dorsal surface of the body and ends in a loop. The free part is about as long as the body.

There is no clear evidence of the existence of a distinct undulating membrane.

The details of the dimensions of the trypanoplasm are given in table 3.

Table 3. Body dimensions of *T. vidyai* sp. nov. from *M. seenghala* (based on 50 forms).

Particulars (μm)	Minimum	Maximum	Average
Length of cell body	15.53	30.13	19.16
Width of cell body	7.53	15.53	11.84
Length of nucleus	4.70	9.41	6.76
Width of nucleus	1.88	5.64	3.15
Length of kinetoplast	2.82	6.82	4.78
Width of kinetoplast	0.70	3.29	1.43
Length of anterior free flagellum	20.24	30.60	25.05
Length of posterior free flagellum	11.77	26.36	18.06

This is the first trypanoplasm to be described from *M. seenghala* and the fifth from fishes of the genus *Mystus*. A comparison of this species with the other four as well as the other species described from various other fresh water fishes show it to be distinct.

It has a relatively short, broad and stumpy body as contrasted with *T. indica* ($19.16 \times 11.84 \mu\text{m}$ as against $28.5 \times 8.0 \mu\text{m}$). The kinetoplast is shorter and broader than in *T. indica* and the flagella distinctly longer. The presence of a distinct kinetoplast and a definite body shape distinguish this species from *T. Krishnamurthyi* sp. nov. Compared with *T. cavacii* sp. nov. it is distinctly larger with a more stumpy appearance because of its broad body. It also has a larger nucleus which is more spherical or ovoid and clearly longer flagella than the latter. Its smaller size, typical and almost constant body shape, much larger kinetoplast and distinctly longer and delicate flagella, distinguishes this species from *T. mysti*.

Its monomorphic nature, longer flagella and the shape and size of the kinetoplast differentiate it from *T. atti* and *T. qadrii* Krishnamurthy and Wahul (under publication). The shape and size of its body, the short and broad nature of its kinetoplast and long flagella demarcate this species from the others described from this area, namely *T. saranae*, *T. lomi* and *T. solapurensis* Wahul (under publication).

The broad and stumpy body of this species and the nature of its kinetoplast distinguish this species from all the others described so far.

The species under discussion is unique in having a posterior flagellum running very close to the dorsal surface throughout the length of the body and in having the free trailing part recurving forwards and often forming a loop.

In view of these differences it is considered new and designated *Trypanoplasma vidyai* sp. nov.

3.4 *Trypanoplasma seenghali* sp. nov. figures 16–20

Host: *Mystus seenghala* Sykes.

Locality: River Purna, Parbhani Dist., Maharashtra, India.

Site of infection: Blood.

This trypanoplasm was found in only one fish (*Mystus seenghala*) out of the 28 examined. This fish also harboured another species of *Trypanoplasma* (*T. vidyai* sp. nov.) and a species of *Trypanosoma*.

The infection was light in the case of the present species.

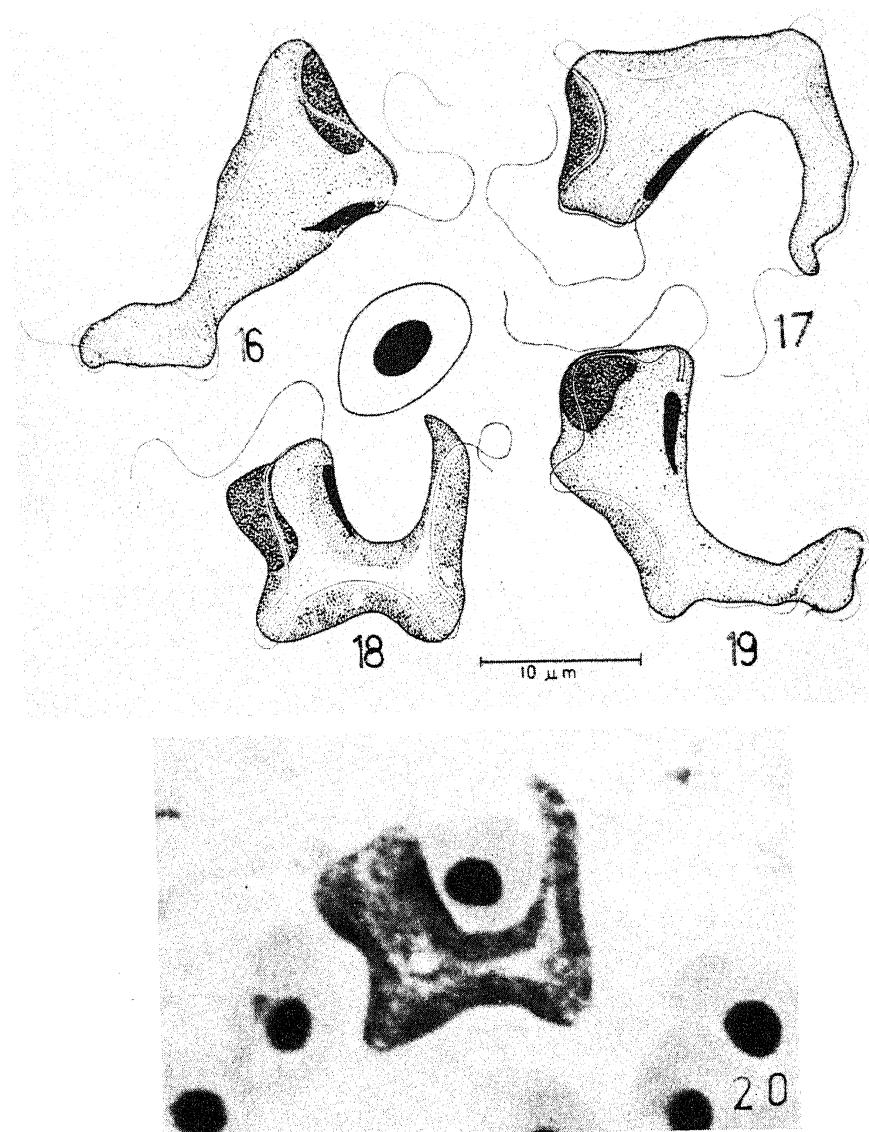
Morphology

Cell body: The body of the trypanoplasm is elongated ($L:W = 3:1$) with a typical 'C' (figure 19) or 'U' (figure 18) shaped configuration. The anterior third is almost twice as broad as the posterior third. The anterior end is bluntly conical and the posterior end tapering to a rounded tip.

Cytoplasm: The cytoplasm is vacuolated, granular and stains homogeneously.

Nucleus: The nucleus is oval (figure 19) to elongated (figure 16) and situated dorsally in the anterior third.

Kinetoplast: The kinetoplast is elongated ($L:W = 6:1$) apparently straight, stiff and rod like (figures 17, 18). The anterior half is broader than the posterior. The posterior end tapers to a point and runs along or close to the ventral margin.



Figures 16-20. *Trypanoplasma seenghali* sp. nov. from *M. seenghala*. 16-19. Camera lucida drawings. 20. Photomicrograph.

Flagella and undulating membrane: The two delicate flagella arise from the kinetosomes, which are placed just anterior to the kinetoplast. The anterior flagellum is almost as long as the body. The posterior flagellum runs along the dorsal surface and becomes free posteriorly. The free trailing part of the posterior flagellum is about three fourths of the body length. During its course the posterior flagellum is thrown into two conspicuous folds, one near the junction of the anterior and middle thirds of the body and the other near the junction of the middle and posterior thirds (figures 17, 18) and in some cases two or three very small folds in the posterior third (figures 17, 19).

There is no clear evidence of a distinct undulating membrane.

The details of dimensions of the trypanoplasm are given in table 4.

This is the second species of trypanoplasm to be described from this host species (i.e. *M. seenghala*). Though it is described from the same host as *T. vidyai* sp. nov., it is easily marked off from that species by conspicuous differences in the shape and dimensions of

Table 4. Body dimensions of *T. seenghali* sp. nov. from *M. seenghala* (based on 25 forms).

Particulars (μm)	Minimum	Maximum	Average
Length of cell body	15.53	32.95	25.27
Width of cell body	5.17	10.82	8.00
Length of nucleus	3.76	9.88	6.93
Width of nucleus	1.41	4.23	2.80
Length of kinetoplast	3.76	8.00	5.70
Width of kinetoplast	0.47	1.88	0.98
Length of anterior free flagellum	20.71	37.30	23.69
Length of posterior free flagellum	12.24	25.42	17.84

the body, the different nature of the kinetoplast and the pattern of undulations of the posterior flagellum. Its characteristic shape, typically elongated kinetoplast and longer flagella contrast it from *T. indica*. Its distinctly larger size and presence of a kinetoplast differentiate it from *T. Krishnamurthyi* sp. nov. which is smaller and lacks a kinetoplast. It differs from *T. cavacii* sp. nov. being larger in size and having a relatively elongated and narrow kinetoplast.

It is marked off from *T. mysti* by its distinctly longer kinetoplast, larger nucleus and conspicuously longer flagella.

Its monomorphic nature marks it off from *T. atti* and *T. qadrii* Krishnamurthy and Wahul (under publication), while its characteristic shape, kinetoplast and body dimensions distinguish it from all the species described so far from this area. This is the largest of the trypanoplasms found during the present study and the only species which is close to this in body dimensions is *T. solapurensis* Wahul (under publication) which has a length range of $16.94 \times 28.24 \mu\text{m}$ as compared with $15.53 \times 32.95 \mu\text{m}$ here. However, the present species is much broader and differentiated from that species by the shape and size of the nucleus and kinetoplast and distinctly longer flagella.

Compared with the various other monomorphic trypanoplasms described so far, it has an overlapping length range with *T. abramidis* and *T. barbi*. However, its body is broader than that of *T. barbi* and narrower than that of *T. abramidis*. It also has a kinetoplast which is shorter than in the two species and a trailing flagellum which is distinctly much longer, besides slight differences in the size, shape and position of the nucleus.

Its body dimensions mark it off from the rest of the species.

In view of the above discussion, this species is considered new and designated *Trypanoplasma seenghali* sp. nov. after the specific name of the host.

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